



**Karolinska  
Institutet**

**Department of Biosciences and Nutrition**

# Spatio-temporal modeling of morphology and gene-expressions during *C. elegans* embryogenesis using a new imaging framework

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
Institutet offentligen försvaras i Röda seminarierummet, Plan 6,  
Novum

**Onsdagen den 11 april, 2012, kl 13.00**

av

**Johan Henriksson**

*Huvudhandledare:*

Thomas R Bürglin  
Karolinska Institutet  
Department of Biosciences and nutrition

*Bihandledare:*

Staffan Strömblad  
Karolinska Institutet  
Department of Biosciences and nutrition

*Fakultetsopponent:*

Jason Swedlow  
University of Dundee  
Wellcome Trust Centre for Gene Regulation and  
Expression

*Betygsnämnd:*

Laszlo Szekely  
Karolinska Institutet  
Department of Microbiology, Tumour and Cell biology

Simon Tuck

Umeå university  
Umeå Centre for Molecular Medicine (UCMM)

Stefan Carlsson  
KTH  
NADA

**Stockholm 2012**

## ABSTRACT

Many aspects of ontogenesis, the process whereby an organism develops from the fertilized egg to a grown adult, are still poorly understood, despite having been studied since Aristotle's time. The advent of modern biology with the sequencing of entire genomes and mapping of all genes offers the promise that one day we will understand how genes function to regulate cell division and differentiation. These advances offer new possibilities to solve the question that humanity has been asking since the beginning of time, how is a new life created?

The aim of this thesis is to establish methods to study when and where particular genes are expressed in the model organism *Caenorhabditis elegans* by means of microscopy. Ultimately, this will aid our understanding of embryology in more complex organisms and in human. The usefulness of the software is however not limited to embryology but is also useful for microscopy in general.

In Paper I we present our open source software platform, Endrov (<http://www.endrov.net>), for image processing and microscopy. It is strongly modular and meant to allow tight integration with all kinds of infrastructure (microscopes, cameras, controllers, etc.) needed for modern biological and non-biological imaging. Endrov supports modern visualization, over 100 file formats, can connect to the OMERO image server and can control many modern light microscopes. We have also implemented 140 image processing algorithms and several types of annotations, e.g., for particle tracking, 3D surfaces, point-text annotation, neuron/vascular 4D annotation and more. A novel feature is the concept of laziness, which makes Endrov scale for large data sets and consequently makes it easier to prototype algorithms. The entire application is written in Java and consists of about 150 000 lines of code.

In Paper II we have developed procedures to record wild-type *C. elegans* embryos in 3D over time (4D). Endrov was used to analyze the data and we generated a model of normal embryonic development complete up to the 150 cell-stage. The variance in cell positioning and division timing was calculated and the cell-cell contacts estimated. When compared to a previous model made from a squeezed embryo, our model has a higher time resolution and is more reproducible.

In Paper III we have generated a revised list of all homeobox genes in *C. elegans*, showing that out of 103 homeobox genes, 70 are co-orthologous to human homeobox genes. A number of modules have been developed for analyzing and quantifying expression data. These are not only suitable for *C. elegans* but can also be used for other biological model systems. Special features include automatic adjustment of exposure time during recording resulting in an increased dynamic range beyond the limits of the microscope camera, and annotation of the cells in 3D rendered space. We have applied the new framework to examine homeobox gene expression patterns and provide an analysis of the patterns that we have recorded.

In Paper IV we have extended the applicability of Endrov by using it to study the mitochondrial polymerase gamma (*polg-1*) in *C. elegans*. *polg-1* deletion mutant alleles were analyzed for phenotypes using methods such as measuring lifespan and brood size, qPCR for mtDNA content and transcript levels, and light and TEM microscopy. The main findings were that homozygous *polg-1* mutant animals develop normally, although later in the adult stage they exhibit compromised gonadal function, sterility, as well as reduced viability due to rupture at the vulva. 3D modeling of the gonad revealed structural abnormalities in the germline. Further, the few descendants that are generated are severely compromised and die during embryogenesis. We can deduce that while mtDNA copy number is a limiting factor for development, the maternally contributed mitochondria are enough to sustain development to adulthood.

In summary, we have created the Endrov software framework that allows processing of large multidimensional microscopy image data sets and demonstrated that it can be applied to a wide range of problems.